Discussion: Immunization with an avirulent live *S choleraesuis* vaccine has demonstrated consistent clinical and bacteriologic control of *S choleraesuis* infections in swine. Clinical signs, including febrile response, diarrhea and mortality, along with growth rate, are significantly improved ($p<0.05$). Pigs from one day of age and older may be safety vaccinated intranasally, or orally, via drinking water, against disease associated with *S choleraesuis*, and to reduce the culture prevalence and shedding of other serovars of Salmonellae (Nolan et al., 2000). Additionally, levels of the organism are effectively reduced in vaccinated, challenged pigs. Fecal shedding of *S choleraesuis* is significantly lower in vaccinated challenged pigs as compared to challenged control pigs. Organ culture prevalence is likewise dramatically lowered in vaccinated pigs. Vaccination at one day of age is at least as efficacious, and may offer even greater advantage over non vaccinated controls.

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O 77 Reduction of salmonella contamination in pork carcasses by vaccination

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Summary: Two field studies and one laboratory challenge were performed to evaluate of vaccination with a live, avirulent *Salmonella choleraesuis* vaccine (Enterisol® SC-54) to provide cross protection, reduce the level of internal culture and fecal shedding of multiple serovars of *Salmonella*. Barns of grow-finish pigs were vaccinated orally, via drinking water, or left as matched controls in the field studies. Ileocecal lymph nodes and spiral colon fecal material were collected at the abattoir from field studies. Three week old pigs were vaccinated intranasally and challenged at five weeks of age in the laboratory study. Internal organ culture and fecal shedding were measured two weeks following challenge with *S typhimurium*. In all three studies, vaccinated pigs had significantly lower culture prevalence of non *S choleraesuis* serovars ($p<0.05$), and reduced fecal shedding in following laboratory challenge ($p<0.05$). A nominal ($p=0.07$) trend to improved growth rate following laboratory challenge with *S typhimurium* challenge was also detected.

Keywords: prevalence, shedding, food safety, Enterisol® SC-54, performance

Introduction: *Salmonella* infections in pork have been linked to outbreaks of food born disease in humans, attracting attention to those strategies that may help reduce *Salmonella* in pork (Letellier et al., 2001). Those practices that reduce the level of contamination or carriage of *Salmonella* in carcasses that arrive at abattoirs should assist in reducing *Salmonella* contamination of post-harvest pork products. Enterisol® SC-54 (Boehringer Ingelheim Animal Health GmbH), an avirulent live *Salmonella*

choleraesuis vaccine, has been utilized successfully to control clinical disease associated with *Salmonella choleraesuis* in pigs (Roof et al., 1992). The purpose of these studies was to demonstrate reduction of *Salmonella* shedding and culture prevalence in carcasses (Baum, 1997) and reduction of sero-prevalence at slaughter (Nolan et al., 2000) in commercial production systems, and following a laboratory challenge. These studies included reduction of *Salmonella* contamination of carcasses at harvest in two conventional production systems, and a reduction in culture and shedding *S typhimurium* in a laboratory challenge.

Materials and Methods: In two large field studies (>50,000 pigs), grow-finish pigs from two production systems were randomly assigned at placement to finishing into either vaccinated or control groups (barn = Exp. Unit). Control and vaccinated barns were matched by animal source, feed source, field management, barn type and season. In most cases control barns were housed at the same location as vaccinated pigs. Vaccine was administered orally, via drinking water, at placement to grow-finish. Facilities were managed in an all in/all out fashion. Total slat, confined finishing barns were used for all groups in study one. Study two utilized partially slatted finishing barns. Other variables that might affect *Salmonella* contamination, e.g. feed type and source, pig source, pre-harvest handling, etc, were consistent between each group (Dahl et al., 1996). Each treatment consisted of 12 or more groups of animals in each study.

A random sample of 35 pigs from each group were selected from either the middle (study one) or last marketing group (study two), and transported by clean, disinfected livestock trailers to the abattoir. Pigs were held for a maximum of three hours prior to harvest. Power calculations for selecting sample size were based on alpha <0.05 and beta >0.8 statistical criteria.

Pig identity was established, and viscera sets (lung, liver, intestines) were individually bagged in clean plastic bags. The viscera were removed to a separate clean room at the facility for collection of ileocecal lymph nodes (study one and two) and spiral colon fecal content (study two only). Additionally, blood and diaphragmatic samples were collected from a random subset of pigs. Culture processes and organ selection followed previously described methods (Hurd et al., 2001). Field collection and laboratory personnel were blinded to source (vaccinated or control) during culture, serotyping and external identification of serovar. The Danish Mix ELISA test was utilized to determine the correlation of group culture status vs. serologic status.

In the laboratory challenge, three week old pigs were obtained from a farm with no history of clinical Salmonellosis in nursery age animals. Pigs were blocked by weight and sex, and randomly assigned to vaccinated (n=20), strict control (10) or challenge control (10) groups. Vaccinated pigs received one dose of Enterisol® SC-54 intranasally, while challenge controls received diluent placebo. Pigs were maintained for two weeks in isolated rooms on wire flooring prior to oral challenge with virulent *S typhimurium* to allow for development of immunity. Fecal culture was performed in all groups both prior to and during the challenge study period.

Vaccinated and challenge control pigs were challenged orally with $>10^8$ virulent *S typhimurium*. Pigs were evaluated for clinical signs, weight gain and fecal shedding for a period of two weeks following challenge. At the end of the two week period, animals were humanely euthanized and internal organs (lung, liver, spleen, lymph node) and fecal samples collected for bacteriologic culture. Methods utilized have been previously described (Hurd et al., 2001).

Results: Vaccinated pigs demonstrated a statistically significant reduction in the percent of carcasses culture positive for *Salmonella* at slaughter in both field studies. In study one, vaccinated pigs had 12% of ileocecal lymph nodes culture positive for *Salmonella* species as compared to 24% of non vaccinated pigs ($p<0.05$). On serologic examination, 14% of vaccinated pigs were seropositive on

the Mix-ELISA, significantly less than the 26% of seropositive control pigs ($p<0.05$). Individual serum samples were considered positive on Mix-ELISA at OD % 40 or above.

In study two, vaccinated groups of pigs averaged 7.4% culture positive in ileocecal lymph nodes, as compared to 26.8% in non vaccinated controls, again a significant reduction ($p=0.03$). Prevalence in non-vaccinated control pigs was similar to other surveys in both studies (Hurd, 2002). Reduction was significant for all *Salmonella* species, and non *S choleraesuis* species as a group. *S typhimurium* and *S derby* were the most commonly isolated organisms from the ileocecal lymph nodes. Vaccinated groups averaged 10.2% seropositive, while non vaccinated control groups had a mean of 24% seropositive. Due to a wide range of seroprevalence in controls (sd=31%), the difference between groups was not significant at the sample size utilized ($p>0.1$).

A trend toward reduction of *Salmonella* prevalence in spiral colon fecal samples was also noted in study two ($p=0.1$). Thirty-two percent of non-vaccinated pigs had *Salmonella* detected in fecal samples, as compared to 12.9% in vaccinated pigs. The relatively small number of study groups (only 10 groups of each treatment were collected) compared to the high variability in control group fecal culture prevalence (sd = 37.5%) suggests insufficient power in the fecal sample portion of the study. A sample size of 43 groups would be required for alpha <0.05 with the same means, and the same standard deviation in the control pigs. *S typhimurium* and *S derby* were the most commonly isolated organisms from spiral colon fecal samples.

The correlation r^2 of group culture status vs. serologic status was 0.67. Culture positive was defined as one or more ileocecal lymph nodes culture positive. Seropositive groups were defined as one or more serum samples positive on Mix-ELISA (OD % 40 or above). One group of pigs was 57% culture positive (13 positive for *S derby,* 2 for *S anatum* and 1 *S typhimurium*) at slaughter but was seronegative. Pen fecal samples collected prior to harvest of this group contained *S derby* and *S typhimurium*, suggesting a late finishing exposure to *S derby.* This demonstrates the limits of categorization on the basis serology using a point in time or intermittent basis, as compared to utilizing serology as part of an ongoing sampling of a population of animals.

In the laboratory challenge study, at no point in time did strict (non challenged) controls have detectable *Salmonella* in fecal or organ culture, indicating effect control of unintentional exposure. Only three of twenty vaccinated pigs had detectable *S typhimurium* in organs or feces at post mortem 14 days following challenge. In contract, all 10 control pigs were positive. Thirteen of 20 vaccinated pigs shed *S typhimurium* for one or more days during the study period, while seven vaccinated pigs did not have detectable shedding. All ten control pigs shed *S typhimurium* for at least one day during the study period. Both measures showed significant ($p<0.05$) reduction in *S typhimurium* in organs or feces in pigs receiving a live avirulent *S choleraesuis* vaccine. These findings are consistent with those of Baum (1997) that demonstrated an association of *Salmonella* exposure with reduced performance.

Vaccinated pigs did not seroconvert on the Danish Mix-ELISA. Subsequent examination of serum samples using a commercial Salmonella LPS ELISA (Idexx Laboratories, Westbrook, Maine) has shown similar lack of seroconversion in pigs vaccinated with Enterisol® SC-54. One possible explanation for this phenomenon is the lack of the *vpl* virulence plasmid in the vaccine isolate (Kennedy et al., 1999). Regardless the mechanism, the ability to utilize a commercial serologic kit to evaluate exposure to field *Salmonella* in vaccinated animals would be a valuable epidemiologic tool.

Discussion: Several studies have suggested that vaccination with an avirulent live *Salmonella* vaccine may allow for cross protection and reduction of *Salmonella* in several species (Fox et al., 1997; House et al., 2001). The results of the three independent studies described further support the following conclusions regarding vaccination of pigs with Enterisol® SC-54:

a.) Vaccination reduces *Salmonella* prevalence in carcasses at harvest.
b.) Reduction occurs in all species of *Salmonella*, not just homologous/Group C1 *Salmonellae*.
c.) Vaccination can be a valuable tool in reducing risk of foodborne disease due to *Salmonella* in pork. Vaccination will not interfere with serologic categorization of farms' *Salmonella* status.

Vaccination may be considered as another potential tool for improving safety of pork by means of reducing the level of salmonella contamination of pork carcasses, and potentially ground or other fresh pork products. Commercial serologic tests may be utilized in conjunction with vaccination to evaluate the success of *Salmonella* reduction programs in swine.

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QUANTIFYING TETRACYCLINE RESISTANCE O 78

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Summary: This study's goal was to evaluate the impact of sub-therapeutic feeding of chlortetracycline (CTC) on the fecal concentration of tet(C), a gene that confers tetracycline resistance via an efflux mechanism. We developed a real-time quantitative PCR assay to measure the quantity of tet(C) in whole fecal DNA samples. The vast proportion of variability in tet(C) (91%) was associated with differences in concentration between the individual pigs, and there was no significant difference in the copy number of tet(C)/mg of feces between the treatment and control pigs ($p>0.05$, linear regression, SPSS 11.0.5)
Keywords: real-time quantitative PCR, tet(C)
Introduction: Antimicrobial resistance (AR) research has been predominantly confined to the study